

What is claimed is:

1. A cartridge for sensing at least one analyte in a sample, said cartridge comprising:

a sample holding chamber for receiving said sample and retaining said sample;

a first conduit connected to said sample holding chamber;

5 at least one analyte sensor, wherein said sensor comprises an analyte-responsive surface and said surface is within said first conduit;

a second conduit for retaining a fluid, said second conduit connected to said first conduit;

means for inserting at least one air segment into said first or second conduit; and

10 pump means capable of displacing said sample from said holding chamber into said first conduit, said pump means further capable of displacing said fluid from said second conduit into said first conduit.

2. A cartridge for sensing at least one analyte in a sample, said cartridge comprising:

15 a sample holding chamber for receiving said sample and retaining said sample;

a first conduit connected to said sample holding chamber;

at least one analyte sensor, wherein said sensor comprises an analyte-responsive surface and said surface is within said first conduit;

a second conduit for retaining a fluid, said second conduit connected to said first conduit;

20 a valve connected to an opening in said first conduit, wherein said valve is closed by contact with said sample; and

pump capable of displacing said sample from said holding chamber into said first conduit, said pump further capable of displacing said fluid from said second conduit into said first conduit.

5 3. A cartridge for sensing at least one analyte in a sample, said cartridge comprising:

a sample holding chamber for receiving said sample and retaining said sample;

a first conduit connected to said sample holding chamber;

10 at least one analyte sensor, wherein said sensor comprises an analyte-responsive surface and said surface is within said first conduit;

a second conduit for retaining a fluid, said second conduit connected to said first conduit;

15 a valve connected to an opening in said first conduit, wherein said valve is closed by contact with said sample;

means for inserting at least one air segment into said first or second conduit; and

pump capable of displacing said sample from said holding chamber into said first

15 conduit, said pump further capable of displacing said fluid from said second conduit into said first conduit.

20 4. The cartridge as in claim 1, 2, or 3, further comprising at least one sensor capable of detecting an air-liquid interface.

5. The cartridge as in claim 1, 2, or 3, wherein said cartridge is single-use.

6. The cartridge as in claim 1 or 3, wherein said segment is a single segment or a plurality of segments.

7. The cartridge as in claim 1 or 3, wherein said segment is inserted into said first 5 conduit.

8. The cartridge as in claim 1 or 3, wherein said segment is inserted into said second conduit.

9. The cartridge as in claim 4 wherein said at least one sensor capable of detecting 10 an air-liquid interface is a conductimetric sensor.

10. The cartridge as in claim 1, 2, or 3, wherein said cartridge further comprises a metering means for delivering a metered amount of said sample to said at least one analyte 15 sensor.

11. The cartridge as in claim 10 wherein said metering means comprises a capillary stop within said first conduit.

20 12. The cartridge as in claim 1 or claim 3 wherein said means for inserting at least one air segment is selected from the group consisting of an air sac comprising a pneumatic means for displacing air from said air sac into said second conduit, a dry chemical that produces a gas when dissolved, a plurality of electrolysis electrodes operably connected to a current

source, a porous means that absorbs fluid displacing air from said porous means into said conduit, and a vent that permits an air segment to enter the fluid when said pump means moves said fluid.

5 13. The cartridge as in claim 2 or claim 3 wherein said closeable valve is a dry sponge material coated with a fluid impermeable layer, a flap capable of blocking said valve and held open by a dry soluble compound or a gelling polymer.

10 14. The cartridge as in claim 1, 2, or 3, further comprising at least one constriction to control fluid flow within said first and second conduits.

15 15. The cartridge as in claim 1, 2, or 3 wherein said second conduit further comprises a valve responsive to hydrostatic pressure.

16. The cartridge as in claim 15, wherein said valve comprises a constriction in said conduit, wherein said constriction has a fluid-contacting surface comprising a hydrophobic surface.

17. The cartridge as in claim 1, 2, or 3, further comprising a third conduit connecting 20 said second conduit and an overflow chamber.

18. The cartridge as in claim 1, 2, or 3, wherein said pump is selected from the group consisting of an air sac contacting a pneumatic means whereby pressure is applied to said air sac, a flexible diaphragm, a piston and cylinder, an electrodynamic pump, and a sonic pump.

5 19. The cartridge of claim 1, 2, or 3, in which said analyte-responsive surface comprises an antibody.

10 20. The cartridge as in claim 1, 2, or 3, wherein a portion of at least one conduit further comprise at least one dry reagent capable of dissolving in said fluid or sample.

15 21. The cartridge as in claim 20 wherein said at least one dry reagent is an antibody-enzyme conjugate, a substrate for said antibody-enzyme conjugate, or a blocking agent.

22. The cartridge as in claim 21 wherein said at least one antibody-enzyme conjugate comprises an enzyme that is urease, glucose oxidase, peroxidase, or alkaline phosphatase.

23. The cartridge as in claim 22 wherein said substrate is urea, glucose, hydrogen peroxide, or a molecule having a phosphate or peroxide moiety.

20 24. The cartridge as in claim 1, 2, or 3, wherein said at least one analyte sensor is an immunosensor.

25. The cartridge as in claim 1, 2, or 3 wherein said fluid comprises a substrate for an antibody-enzyme conjugate.

26. The cartridge as in claim 25 wherein said substrate is cleaved to produce an
5 electroactive product.

27. The cartridge as in claim 26 wherein said substrate is a ferrocene or p-aminophenol phosphate.

28. The cartridge as in claim 1, 2, or 3, wherein said at least one analyte sensor is an amperometric sensor, a potentiometric sensor, or a reference sensor.

29. The cartridge as in claim 1, 2, or 3, further comprising a plurality of mechanical and electrical connections for insertion of said cartridge into a reading apparatus.

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30. The cartridge as in claim 1, 2, or 3, wherein said at least one analyte sensor is formed on a substantially planar surface.

31. The cartridge as in claim 1, 2, or 3, further comprising a surface coating that
20 decreases non-specific binding of a substance therein.

32. The cartridge as in claim 1, 2, or 3, further comprising an enzyme and a substrate capable of regenerating a product consumed by contact with said at least one analyte sensor, whereby a signal from said sensor is increased.

5 33. The cartridge as in claim 32 wherein said enzyme is glucose oxidase and said substrate is D-glucose.

10 34. The cartridge as in claim 1, 2, or 3, further comprising mobile microparticles capable interacting with said analyte and further comprising means for localizing said microparticles to said at least one sensor.

15 35. The cartridge as in claim 34, wherein said microparticles are magnetic, and said means for localizing said microparticles is a magnet field.

36. The cartridge as in claim 34, further comprising a filter element interposed between said sample holding chamber and said at least one analyte sensor, and adjacent said at least one sensor, whereby said microparticles are concentrated adjacent said at least one sensor.

20 37. An analyte sensor of claim 1, 2 or 3 comprising a microfabricated base sensor with a bioactive layer, superimposed over at least a portion of said base sensor, said layer comprising a sufficient amount of a biomolecules attached thereto, wherein said biomolecule is sensitive to said analyte.

38. The microfabricated sensor as in claim 37 in which said base sensor comprises an amperometric electrode.

39. The microfabricated sensor as in claim 37 further comprising a ground electrode.

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40. The microfabricated sensor as in claim 37 further comprising a reference electrode.

41. The microfabricated sensor as in claim 37 wherein said biomolecule is an antibody or fragment thereof.

42. The microfabricated sensor as in claim 37 wherein said biomolecule is selected from the group consisting of ionophores, cofactors, polypeptides, proteins, glycopeptides, enzymes, immunoglobulins, antibodies, antigens, lectins, neurochemical receptors, 15 oligonucleotides, polynucleotides, DNA, RNA, and mixtures thereof.

43. The microfabricated sensor as in claim 37 wherein said biomolecule is an antibody capable of binding an analyte selected from the group consisting of human chorionic gonadotrophin, troponin I, troponin T, troponin C, a troponin complex, creatine kinase, creatine 20 kinase subunit M, creatine kinase subunit B, myoglobin, myosin light chain, fragments thereof, and modified fragments thereof.

44. The microfabricated sensor as in claim 43 wherein said modified fragments are generated by oxidation, reduction, deletion of at least one amino acid, addition of at least one amino acid, chemical modification with a natural moiety, or chemical modification with a synthetic moiety.

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45. The microfabricated sensor as in claim 38, wherein said biomolecule has an affinity constant for said analyte ligand of greater than about 10^7 M^{-1} .

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46. A method for detecting at least one analyte in a sample using a sensing cartridge, said method comprising the steps of:

placing said sample into the sample holding chamber of the cartridge of claim 1 or claim 2;

activating said pump means, whereby said sample contacts said at least one analyte-responsive surface;

activating said pump means whereby said sample is displaced from contact with said analyte-responsive surface;

activating said pump means to contact said at least one analyte-responsive surface with said fluid; and

recording a response from said at least one analyte sensor, whereby said analyte is
20 detected.

47. A method for detecting at least one analyte in a sample using a sensing cartridge, said method comprising the steps of:

placing said sample into the sample holding chamber of the cartridge of claim 3;

activating said pump, whereby said sample contacts said at least one analyte-responsive surface;

activating said pump whereby said sample is displaced from contact with said analyte-
5 responsive surface;

activating said pump to contact said at least one analyte-responsive surface with said fluid; and

recording a response from said at least one analyte sensor, whereby said analyte is detected.

48. A cartridge for sensing at least one analyte in a sample, said cartridge comprising:

a sample holding chamber for receiving said sample and retaining said sample;

a first conduit connected to said sample holding chamber;

15 at least one analyte sensor, wherein said sensor comprises an analyte-responsive surface and said surface is within said first conduit;

a first and second vent within said first conduit wherein said sensor is positioned between said sample chamber and said vents;

a second conduit for retaining a fluid, said second conduit connected to said first conduit between said sample chamber and said vents;

20 pump means capable of displacing said sample from said holding chamber into said first conduit and further capable of closing said second vent to retain said sample in the region of said second vent; and

wherein said pump means is further capable of displacing said fluid from said second conduit into said first conduit, and wherein said first vent permits at least one air segment to enter said fluid.

5 49. The cartridge of claim 48, wherein said pump means comprises a variable rate of displacement whereby the volume and number of air segments that enter the fluid is controlled.

50. The cartridge of claim 48, wherein the second conduit comprises a vent.

10 51. The cartridge of claim 48, wherein the sample contacts an adsorbent wicking material in proximity to second vent and is thereby retained and closes said vent.

52. The cartridge of claim 48, wherein the sample chamber further comprises a sample-metering element for providing a predetermined volume of sample to said first conduit.

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53. The cartridge of claim 48, wherein said fluid in said second conduit is initially in a rupturable pouch.

20 54. The cartridge of claims 48 or claim 53, further comprising a reader device, said reader capable (a) of controlling and reading said analyte sensor, (b) of controlling said pump means, and (c) of controlling said rupturable pouch.

55. The cartridge of claim 48, wherein said first vent comprises a gas permeable membrane capable of controlling the rate of air entry through said first vent.

56. The cartridge of claim 1, 2, 3, or 48 wherein said sample holding chamber further comprises a closure means.

57. The cartridge of claim 48, wherein said analyte sensor is an electrochemical immunosensor comprising an electrode having a layer of immobilized first antibody that binds said analyte and further comprising a counter/reference electrode.

58. A method of measuring the amount of an analyte using an electrochemical assay in a conduit comprising a sensor, wherein said sensor comprises an electrode having a surface layer of immobilized antibody that binds said analyte, and a counter/reference electrode disposed within said conduit, said method comprising:

55 contacting said sensor with a liquid sample containing said analyte;

contacting said sensor with an enzyme-labeled antibody capable of binding said analyte, whereby a complex of immobilized antibody, analyte and labeled antibody is formed;

contacting said sensor with a solution comprising a substrate for said enzyme and at least one air segment to remove unbound analyte and labeled antibody from said sensor region;

20 removing substantially all said fluid from said sensor while retaining said fluid over said electrode, said counter/reference electrode, and a contiguous portion of the wall connecting said electrodes; and

detecting the product of the reaction between said enzyme and said substrate using said sensor, whereby the amount of analyte in the liquid sample is measured.

59. The method of claim 58, wherein the sample comprises the enzyme-labeled antibody dissolved therein.

5 60. The method of claim 58, wherein the enzyme-labeled antibody is subsequently delivered in a liquid to the sensor that is not the sample, prior to delivery of said fluid.

61. The method of claim 58, wherein the layer retains a predetermined volume of fluid over the electrodes when the fluid is removed from the body of said conduit.

62. A method of claim 58, wherein at least a portion of the wall of the conduit is treated to reduce non-specific binding of the enzyme-labeled antibody.